JOURNAL OF VIROLOGY, June 1973, p. 879-885

Vol. 11, No. 6
Printed in U.S.A.

Prophage Induction by High Temperature in Thermosensitive *dna* Mutants Lysogenic for Bacteriophage Lambda

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Received for publication 14 December 1972

High-temperature treatment of thermosensitive *dna* mutants lysogenic for phage λ leads to prophage induction and release of phage (at the permissive temperature) in elongation-defective mutants of the genotypes *dnaB*, *dnaE*, and *dnaG*. In initiation-defective mutants no prophage induction occurs at 42 C in mutants of the genotype *dnaA*, whereas with a *dnaC* mutant as well as with strain HfrH 252 (map position not yet known) phages are released at 42 C. DNA degradation at the replication fork at 42 C is observed in all *dnaB*(λ) mutants tested, but not in mutants of the genotypes *dnaE*(λ) and *dnaG*(λ). Therefore, degradation of replication fork DNA is not a prerequisite for prophage induction.

In λ-lysogenic bacteria, phage development can be induced by a variety of treatments that damage the bacterial chromosome and inhibit DNA replication. Since the bacterial *recA* function is required for induction (3), it is supposed that induction results from *recA*-mediated repair of damaged DNA. The mechanism of induction is still obscure (5).

In thermosensitive *dna* mutants, DNA replication can be interrupted at high temperature (42 C and above), and the mode of cessation of DNA synthesis varies markedly in the different classes of *dna* mutants. In most of the mutant types known to be defective in chain elongation (*dnaB*, *dnaE*, *dnaG*), DNA synthesis stops immediately after reaching the critical temperature (42 C; reference 23). A residual DNA synthesis at the elevated temperature is observed in mutant types defective in the process of initiation (*dnaA*, *dnaC*, strain HfrH 252; 1, 4, 12). In *dnaB* mutants DNA at the replication fork is degraded extensively at 42 C (19).

It seemed worthwhile to us to examine λ-lysogenic *dna* mutants of different genotypes for their ability to induce the prophage at high temperature in order to find out whether or not a damaged bacterial DNA is a necessary prerequisite for induction to occur. It has already been reported that prophage λ is not induced when DNA synthesis ceases at 42 C in an *Escherichia coli dnaA* mutant. However, induction occurred in this mutant when the cells were UV-irradiated after completion of rounds of replication at 42 C (20). On the other hand, it has been found that prophage λ is induced at high temperature in bacterial mutants with temperature-sensitive but unknown genetic defects (15, 22). Induction of prophage λ has also been inferred from the loss of colony-forming ability of a *dnaB* mutant after temperature shifts to 42 C (21).

MATERIALS AND METHODS

Radioisotopes. Radioisotopes used were ^{3}H-thymidine, 22 to 27 Ci/mmol, and ^{3}H-thymine, 5 Ci/mmol, from The Radiochemical Centre, Amersham, England.

Bacteria. The *E. coli* strains listed in Table 1 were used. All strains were lysogenized by phage λ (wild type) in our laboratory. In addition, strain HfrH 165/70 was lysogenized by phage λ*ind*$^-$. Strain K-12 C600 was used as indicator for λ.

Media. Tryptone broth: 1% tryptone (Difco) and 0.5% NaCl. K medium: 1:1 mixture of double-strength M9 medium and 3% Difco Casamino Acids (27); A medium (7); M9 medium (27). All synthetic media were supplemented with 20 μg of thymine and thiamine per ml. In the case of strain HfrH 252, the media contained 20 μg of proline per ml in addition.

Measurement of induction of prophage λ by temperature shift. Lambda-lysogenic bacteria grown overnight in tryptone broth at room temperature were usually diluted 1:1,000 into K medium and grown at 30 C to about 1×10^8 cells per ml. The temperature of the culture was then raised to 42 C (or 46 C in the case of *dnaE* mutants) and incubation was continued.

TABLE 1. *Escherichia coli strains*[a]

E. coli strain	*dna* locus	Remarks	Reference
HfrH 252	?		1, 17
HfrH 252rev	*dna*+	Selected for growth at 42 C, our laboratory	This paper
PC5	A		4, 23
E508	A		23
CRT46	A		13, 23
HfrH 165	*dna*+	Parental strain of HfrH 165/70	2, 19
HfrH 165/70rev	*dna*+	Selected for growth at 42 C, our laboratory	This paper
HfrH 165/70	B		2, 19
Hfr 100	B		19
PC6	B		4, 11
PC8	B		4, 11
PC2	C		4, 23
PC7	D		4, 23
BT1126	E		24
E486	E		23
E101	F		9, 23
JW185	G	*dna* allele number 308	11
PC3	G		4, 23

[a] Source of *dna* mutants: HfrH 252, HfrH 165, HfrH 165/70, Hfr 100, BT1126 (H. Schaller and F. Bonhoeffer); PC2, 3, 5, 6, 7, 8 (J. Zeuthen); E486, E101, JW185 (J. Wechsler).

Since the time period for optimal induction at elevated temperatures might be different for different *dna* mutants, an analysis as shown in Fig. 1 and 3 was carried out with at least one mutant of each genotype. For this purpose samples were withdrawn at intervals and (i) assayed immediately for surviving bacteria (colony-forming ability at 30 C), induced prophage, and free phage (after treatment with $CHCl_3$); (ii) diluted 1:100 into K medium and incubation continued for 2 to 3 h at 30 C, after which the culture was again assayed for free phage.

In some experiments, K medium was replaced by tryptone broth, M9 or A medium. The number of cells containing an induced prophage were measured by a spot test in order not to overlook induction processes yielding only low phage bursts. A serial dilution of bacteria (in the range of 50 to 2,000 cells per spot) was spotted onto a lawn of K-12 C600 indicator bacteria. Plates were subsequently incubated at 27 to 30 C. Control cells (grown at 30 C) were included in the spot test to correct for the spontaneously induced prophages.

Measurement of the stability of radioactively labeled chromosomal DNA at elevated temperature: (i) pulse labeling (replication fork DNA). To 1 volume of log-phase grown bacteria in K medium at 30 C, 0.03 volume of ^{3}H-thymidine (1 mCi per ml) was added for 50 s, after which the culture was transferred to 42 C (or 46 C in the case of *dnaE* mutants) with simultaneous addition of 0.05 volume of thymidine (10 mg/ml).

(ii) Uniform labeling (preexisting DNA). Bacteria were grown for six to seven generations at 30 C in K medium supplemented with ^{3}H-thymine (10 μCi and 20 μg per ml). At a titer of 10^8 per ml, the cells were harvested by filtration, washed, and resuspended in the same volume of K medium supplemented with 200 μg of unlabeled thymine. After an additional 15 min of incubation at 30 C, the cultures were divided. One part was shifted to 42 C (or 46 C in the case of *dnaE* mutants), and another part was kept at 30 C.

Samples (0.1 ml) were removed successively from the radioactive cultures. Incorporation of radioactive DNA precursors was terminated, and trichloroacetic acid-insoluble radioactivity was determined as described elsewhere (19).

RESULTS

Induction by temperature shift of prophage λ in lysogenic dna mutants: (i) induction of λ-lysogenic initiation-defective mutants. In initiation-defective mutants, λ is able to replicate at temperatures at which host DNA synthesis has come to a halt (13, 17). Here, induction of prophage and release of phages can be followed directly at the elevated temperature.

The initiation-defective mutants *dnaA* E508(λ) and *dnaA* CRT46(λ) do not undergo spontaneous prophage induction after cessation of DNA synthesis at 42 C (20). Another *dnaA* mutant (PC5), isolated by Carl (4), also does not induce λ prophage efficiently. When this strain is incubated for 2 to 3 h at 42 C, at most 4% of the cells are induced (Table 2).

We would conclude from these results that in initiation-defective mutants cessation of host DNA synthesis per se is not a sufficient condition for λ induction to occur. However, this is not true for an initiation-defective mutant of the genotype *dnaC* PC2 (4). With the nonlysogenic strain, the cell number still increases by a factor of 3.3 reaching a plateau after 2 to 3 h at 42 C. Under the same conditions the λ-lysogenic mutant induces the prophage and the cells die (Table 2).

Still another initiation-defective mutant, strain HfrH 252(λ) (whose map location is not yet known), behaves similarly to the strain *dnaC* PC2. In Fig. 1 phage release at 42 C is followed over a period of 4 h in A medium for the mutant as well as for a revertant of this mutant. In both strains, the number of spontaneously induced phages increases as a consequence of the temperature shift to 42 C. The effect is more pronounced with the mutant than with the revertant strain. However, no cell death is

TABLE 2. *Induction of prophage λ in dna(λ) mutants by high temperature*[a]

dna(λ) mutant	*dna* locus	Induction	Fraction of cells induced	Remarks
HfrH 252	?	+		In A medium: see Fig. 1 In K medium: phage bursts >2 after 3 h at 42 C (surviving cell fraction = 0.15)
PC5	A	−	<0.04 (2-3 h 42 C)	
E508		−		Measured by Monk and Gross (20)
CRT46		−		
HfrH 165/70	B	+	0.4-0.7 (2-3 h 42 C)	
Hfr 100		+		Maximal phage release after 1-2 h at 42 C + 3 h at 30 C (see Fig. 2)
PC6		+		
PC8		+		
PC2	C	+		Phage bursts >2 after 3 h at 42 C (surviving cell fraction = 0.03)
PC7	D	−		Slight increase of spontaneously induced prophages at 42 C
BT1126	E	+	0.1-0.4 (1.5 h 46 C)	Phage bursts usually <1
E486		+	0.7-0.9 (1.5-2 h 46 C)	Maximal phage release after 1.5 h at 46 C + 3 h at 30 C
E101	F	−		
JW185	G	+	0.3 (3 h 42 C)	Maximal phage release after 3 h at 42 C + 3 h at 30 C
PC3		−	0.005 (2 h 42 C)	

[a] Induction is regarded as positive, when at least 10% of the mutant cells yield infective centers. The fraction of cells induced is difficult to determine exactly with the strains *dnaC* PC2(λ) and HfrH 252(λ), since in these mutants λ is able to replicate at 42 C. Here phage bursts are expressed as the number of phage released at 42 C per viable cell at time zero, 42 C.

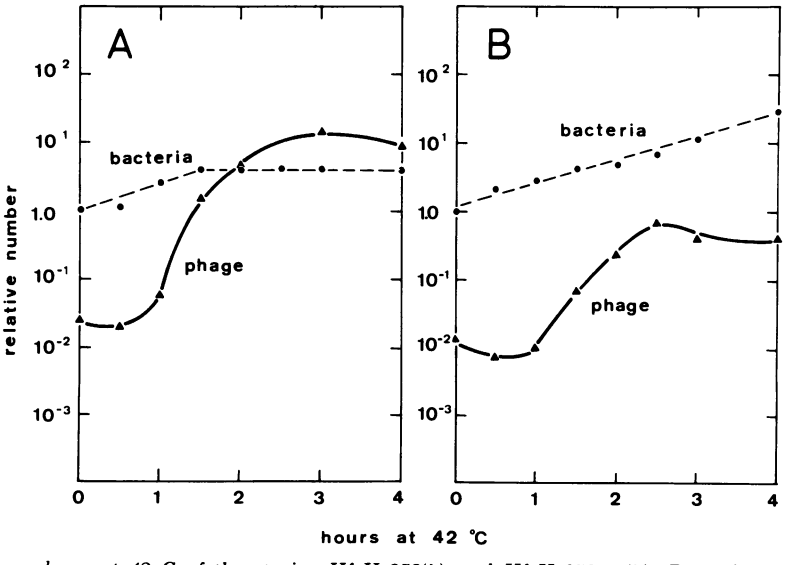

FIG. 1. *Phage release at 42 C of the strains HfrH 252(λ) and HfrH 252rev(λ). Bacteria were grown in A medium at 30 C and incubated in the same medium at 42 C. Cell concentration at time zero, 42 C: A = HfrH 252(λ), 5 × 10^7 per ml; B = HfrH 252rev(λ), 1 × 10^8 per ml.*

observed with strain HfrH 252 after DNA synthesis and cell divisions have come to a halt, indicating that only a minor fraction of cells have been induced. Only the subsequent irradiation with UV light or treatment with mitomycin (both at 42 C) leads to induction (data not shown). However, when K medium was used instead of A medium, the cells died at 42 C and a larger number of phages were released (Table 2).

(ii) Induction of λ-lysogenic elongation-defective mutants. In the elongation-defective mutants investigated, λ DNA is not replicated at the restrictive temperature (6, 13, 17). Therefore, induction of prophage, provoked by the interruption of DNA synthesis, is subsequently measured at temperatures permissive for DNA synthesis. Phage bursts of prophage-induced bacteria are determined at 30 C after the induction period at 42 C.

dnaB mutants. In Fig. 2 phage release at 30 C of strain HfrH 165/70(λ) after an induction period of 100 min at 42 C is shown. A maximal burst is obtained after 3 to 4 h of incubation at 30 C. The phage yield (after 3 h at 30 C) in relation to the preceding induction period at 42 C is shown in Fig. 3A. Largest bursts are obtained after 1 to 2 h of incubation at 42 C, and induction is accompanied by a decrease in the number of surviving bacteria. On the contrary, in the revertant strain, growth at 42 C only leads to a slight increase in the number of spontaneously induced phages (Fig. 3B).

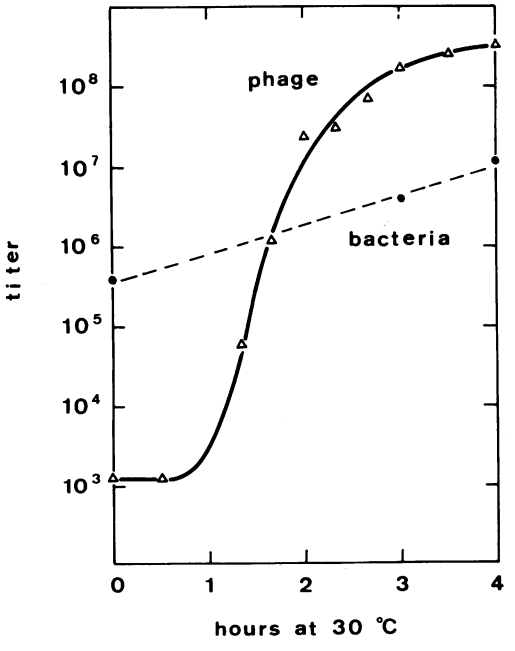

FIG. 2. *Phage release at 30 C of strain HfrH 165/70(λ) after 100 min of preincubation at 42 C. Bacteria were grown in K medium at 30 C and incubated in the same medium at 42 C. Cell concentration at time zero, 42 C: 9 × 10^7 per ml. The culture was diluted 1:100 into K medium after 100 min and incubation was continued at 30 C.*

According to the experimental conditions outlined in Fig. 3, all *dnaB* mutants examined induce λ prophage very efficiently following an incubation period at 42 C of 1 to 2 h (Table 2). However, different *dnaB* mutants differ slightly with respect to the length of time at 42 C required for optimal induction. It is also common to the (lysogenic as well as the nonlysogenic) *dnaB* mutants tested that at 42 C they degrade their DNA at the replication fork extensively (see below and reference 19).

Prophage induction studies were done in more detail with strain HfrH 165/70(λ). Usually 40 to 70% of the cells induced the prophage at 42 C. Induction and phage release in K medium were also very efficient at high concentrations of bacteria (omitting the 1:100 dilution step, see Materials and Methods). By this means phage titers larger than 1×10^{10} per ml were obtained. Prophage induction also occurred when K medium was replaced by tryptone broth. However, for unknown reasons phage yield was extremely low if the 1:100 dilution step (into tryptone broth) was omitted. In M9 medium, phage bursts lower than 0.1 were obtained after 50 min at 42 C and 2 h at 30 C. Prophage λ*ind*$^-$, which is not inducible by UV (14), also cannot be induced by high temperature in 165/70(λ*ind*$^-$).

dnaE mutants. In mutants having a thermosensitive lesion at the *dnaE* locus, DNA polymerase III activity is thermosensitive (10). In the strains E486 and BT1126, DNA synthesis stops at 42 C and 46 C, respectively. No DNA degradation at the replication fork is observed at these temperatures (see below). Both lysogenic strains induce their prophage (Table 2).

dnaG mutants. In the strains JW185 and PC3, the synthesis of short deoxynucleotide chains (Okazaki DNA) is inhibited at 42 C (18). At that temperature no degradation of DNA at the replication fork is observed in strain JW185 (see below and Fig. 4). Strain JW185(λ) induces the prophage at 42 C; strain PC3(λ) does not (Table 2). UV irradiation of the bacteria at 30 C leads to induction of JW185(λ), but PC3(λ) is only poorly induced. As it was found later, phage λ is only weakly reproduced in PC3 at 30 C (in comparison to growth in C600). In this mutant, either the mutation in *dnaG* affects growth of λ at 30 C or the poor growth is due to some other property of the strain.

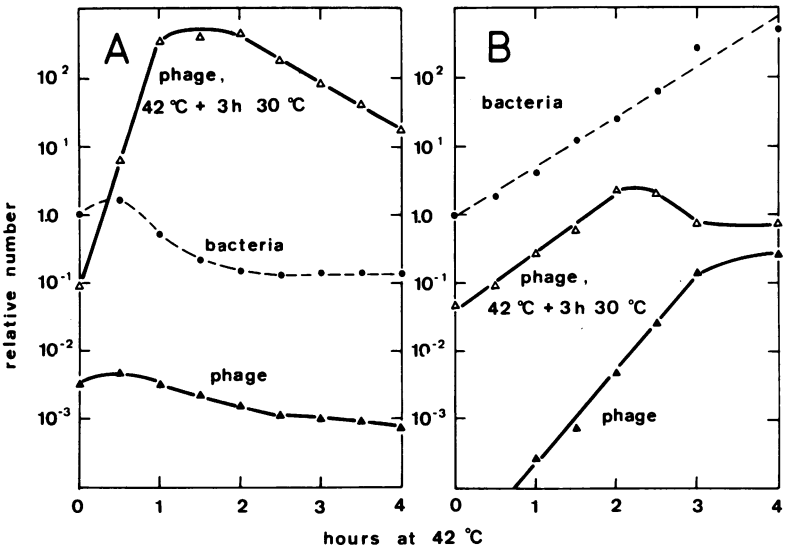

FIG. 3. *Phage release at 30 C after various preincubation periods at 42 C. Bacteria were grown in K medium at 30 C and incubated in the same medium at 42 C. Cell concentration at time zero, 42 C. A = HfrH 165/70(λ), 8 × 10⁷ per ml; B = HfrH 165/70rev(λ), 7 × 10⁶ per ml. Relative number of bacteria (●- - -●) and respective phage (▲——▲) after the indicated time at 42 C. Relative number of phage (Δ——Δ) after the indicated time at 42 C plus an additional time of 3 h at 30 C in each case.*

Stability of chromosomal DNA and λ prophage induction in dna mutants: (i) fate of 30 C pulse-labeled DNA at elevated temperatures. With λ-lysogenic as well as with some nonlysogenic elongation-defective mutants, the effect of high temperature on (i) the replication fork DNA (the most recently made DNA at 30 C) and (ii) the uniformly labeled chromosomal DNA was studied. In the former case the DNA was pulse-labeled with ^{3}H-thymidine immediately before the temperature shift (see Materials and Methods). As was already mentioned above, all the *dnaB* mutants tested (see Table 2) degrade replication fork DNA. As an example, the kinetics of DNA degradation in strain *dnaB* PC8(λ) are shown in Fig. 4. However, when mutants of the genotype *dnaE* [E486(λ) and BT1126] and *dnaG* [JW185(λ) and PC3] were tested in the same way, no significant degradation was observed. The stability of pulse-labeled DNA at 42 C is shown in Fig. 4 for the strains E486(λ) and JW185(λ). Both these mutants, as well as strain BT1126(λ), do induce their prophage by high temperature treatment (Table 2). Obviously degradation of the DNA at the replication fork is not a prerequisite for induction to occur.

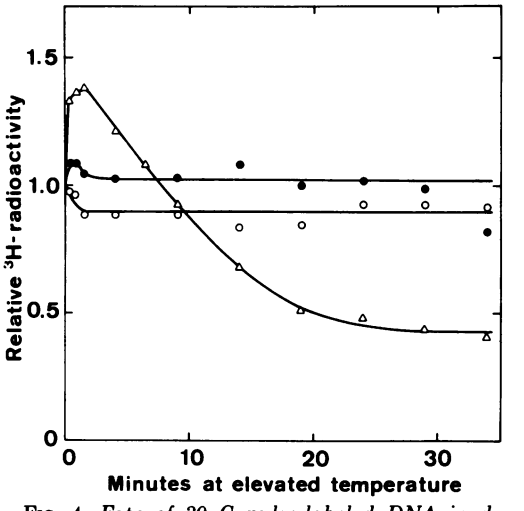

FIG. 4. *Fate of 30 C pulse-labeled DNA in dna mutants at elevated temperatures. Bacteria were grown in K medium at 30 C and pulse labeled with ^{3}H-thymidine at 30 C, and trichloroacetic acid-insoluble radioactivity was followed after temperature shift (see Materials and Methods). Δ, Strain dnaB PC8(λ), 42 C; ○, strain dnaE E486(λ), 46 C; ●, strain dnaG JW185(λ), 42 C.*

(ii) Fate of 30 C uniformly labeled DNA at 42 C. Elongation-defective mutants with ^{3}H-thymine uniformly labeled DNA were incubated in K medium at 30 C and 42 C for 3 h, and trichloroacetic acid-insoluble radioactivity was determined at 30-min time intervals (see also Materials and Methods). With the strains *dnaB* HfrH 165/70(λ), *dnaE* E486(λ), and *dnaG* JW185(λ), between 15 and 20% of the ^{3}H label became acid-soluble after 3 h of incubation at *both* temperatures. However, degradation at 42 C was somewhat faster than at 30 C in all three mutants. Under the same conditions in strain

dna+165(λ) the acid-insoluble ^{3}H label increased by about 5% at 30 C (probably due to a depletion of the residual ^{3}H-labeled precursor pool). These results indicate that in the mutant strains the *dna* defect already leads to a limited turnover of the chromosomal DNA at 30 C. Surprisingly, in strain *dna*+165(λ) 13% of the DNA was also made trichloroacetic acid soluble after 3 h at 42 C. Therefore, it is unlikely that the DNA turnover at 42 C in the *dna* mutants is the reason for induction.

DISCUSSION

Lambda-lysogenic elongation-defective mutants of the genotype *dnaB*, *dnaE*, and *dnaG* induce their prophage at high temperature. Thus, the inactivation of a function responsible for the elongation of a replicating DNA has the same effect as the variety of other treatments which all lead to prophage induction by damaging the DNA and inhibiting DNA replication. Consistent with this are the findings that *dna* mutants with only a reduced DNA synthesis at elevated temperature (*dnaD* PC7, residual DNA synthesis without reaching a plateau [4]; *dnaF* E101, DNA synthesis with an immediate reduction in rate [23] due to a defective ribonucleoside diphosphate reductase [9]) do not induce the prophage (Table 2).

It is striking that all *dnaB* mutants which degrade their DNA at the replication fork at 42 C also induce their prophage and yield large phage bursts. (In comparison to the induction by UV irradiation this "temperature induction" is easier to handle, and we are using it routinely for the preparation of phage stocks.) However, these DNA degradation products are probably not the reason for the release of prophage repression since other elongation-defective mutants (*dnaE*, *dnaG*) which do not degrade their replication fork DNA are also inducible by high temperature. It is also unlikely that a more general degradation or a breakdown and resynthesis of preexisting DNA at 42 C is the reason for induction. Although a turnover of this DNA is observed at 42 C in *dna* mutants, it also takes place in a lysogenic wild type which is not induced at this temperature.

When DNA synthesis is stopped by thymine starvation in *E. coli* strains auxotrophic for thymine, an endonuclease activity appears which introduces nicks into the DNA (8). Since λ prophage can also be induced by thymine starvation (16), nicking of the DNA may trigger in some way the induction process. Such an event could also be the reason for the induction of λ in *dna* mutants. However, preliminary experiments with a (nonlysogenic) *dnaB* mutant ruled out an extensive nicking of chromosomal DNA (19). A more careful analysis of all lysogenic *dna* mutants tested is in progress.

That induction should result from the action of the *recA*-mediated repair of damaged DNA is difficult to explain in the case of such elongation-defective *dna* mutants which do not show a noticeable DNA breakdown but nevertheless induce their prophage (*dnaE* E486, *dnaG* JW185). However, the involvement of the *recA* product in prophage induction may have nothing to do with DNA repair processes. In (nonlysogenic) *dnaB* mutants asymmetric chromosome reinitiations can be triggered by temporary blocks in DNA chain elongation (25). Double mutants of the genotype *dnaB recA*, however, fail to reinitiate chromosome replication after temperature shifts (A. Worcel, M. Schwartz, personal communication). Such abnormal initiations may also trigger prophage induction. They have in fact been observed during induction of a *Bacillus subtilis* prophage (26). It has to be shown whether abnormal chromosomal initiations can be observed also in all inducible elongation-defective *dna* mutants.

In the initiation-defective *dnaA* mutant PC5(λ), the prophage is not induced at 42 C. As was observed earlier, cessation of DNA synthesis at 42 C in two other λ-lysogenic *dnaA* mutants also did not lead to induction (20). Comparing the inducibility of elongation-defective *dna* mutants with the non-inducibility of initiation-defective *dnaA* mutants by temperature shifts, one could assume that the induction process is only triggered in such cells in which the chromosomal DNA is in the process of replication. However, since two other initiation-defective mutants, namely *dnaC* PC2 and HfrH 252 (unmapped), can induce their prophage at 42 C, the explanation cannot be so simple. The fact that the two latter strains behave similarly with regard to induction may indicate that strain HfrH 252 is also a *dnaC* mutant type.

ACKNOWLEDGMENTS

We are much indebted to F. Bonhoeffer, H. Schaller, J. A. Wechsler, and J. Zeuthen for providing us with the *dna* mutants. We would like to thank H. A. Rolton for her assistance in the correction of the manuscript.

LITERATURE CITED

1. Beyersmann, D., M. Schlicht, and H. Schuster. 1971. Temperature-sensitive initiation of DNA replication in a mutant of *Escherichia coli* K12. Mol. Gen. Genet. **111**:145-158.
2. Bonhoeffer, F. 1966. DNA transfer and DNA synthesis during bacterial conjugation. Z. Vererbungsl. **98**:141-149.

3. Brooks, K., and A. J. Clark. 1967. Behavior of λ bacteriophage in a recombination deficient strain of *Escherichia coli*. J. Virol. **1**:283-293.
4. Carl, P. L. 1970. *Escherichia coli* mutants with temperature-sensitive synthesis of DNA. Mol. Gen. Genet. **109**:107-122.
5. Echols, H. 1971. Lysogeny: viral repression and site-specific recombination. Annu. Rev. Biochem. **40**:827-854.
6. Fangman, W. L., and A. Novick. 1968. Characterization of two bacterial mutants with temperature-sensitive synthesis of DNA. Genetics **60**:1-17.
7. Fox, E., and M. Meselson. 1963. Unequal photosensitivity of the two strands of DNA in bacteriophage λ. J. Mol. Biol. **7**:583-589.
8. Freifelder, D., and E. Levine. 1972. Stimulation of nuclease activity by thymine starvation. Biochem. Biophys. Res. Commun. **46**:1782-1787.
9. Fuchs, J. A., H. O. Karlström, H. R. Warner, and P. Reichard. 1972. Defective gene product in *dnaF* mutant of *Escherichia coli*. Nature N. Biol. **238**:69-71.
10. Gefter, M. Y. Hirota, T. Kornberg, J. A. Wechsler, and C. Barnoux. 1971. Analysis of DNA polymerases II and III in mutants of *Escherichia coli* thermosensitive for DNA synthesis. Proc. Nat. Acad. Sci. U.S.A. **68**:3150-3153.
11. Gross, J. D. 1972. DNA replication in bacteria. Curr. Top. Microbiol. Immunol. **57**:39-74.
12. Hirota, Y., J. Mordoh, and F. Jacob. 1970. On the process of cellular division in *Escherichia coli*. III. Thermosensitive mutants of *Escherichia coli* altered in the process of DNA initiation. J. Mol. Biol. **53**:369-387.
13. Hirota, Y., A. Ryter, and F. Jacob. 1968. Thermosensitive mutants of *E. coli* affected in the process of DNA synthesis and cellular division. Cold Spring Harbor Symp. Quant. Biol. **33**:677-693.
14. Jacob, F., and A. Campbell. 1959. Sur le système de répression assurant l'immunité chez les bactéries lysogènes. C. R. Acad. Sci. **248**:3219.
15. Kirby, E. P., F. Jacob, and D. A. Goldthwait. 1967. Prophage induction and filament formation in a mutant strain of *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **58**:1903-1910.
16. Korn, D., and A. Weissbach. 1962. Thymineless induction in *Escherichia coli* K12(λ). Biochim. Biophys. Acta **61**:775-790.
17. Lanka, E., and H. Schuster. 1970. Replication of bacteriophages in *Escherichia coli* mutants thermosensitive in DNA synthesis. Mol. Gen. Genet. **106**:274-285.
18. Lark, K. G. 1972. Genetic control over the initiation of the synthesis of the short deoxynucleotide chains in *E. coli*. Nature N. Biol. **240**:237-240.
19. Mikolajczyk, M., and H. Schuster. 1971. The fate of newly made DNA in *Escherichia coli* mutants thermosensitive in DNA synthesis. Mol. Gen. Genet. **110**:272-288.
20. Monk, M., and J. Gross. 1971. Induction of prophage λ in a mutant of *E. coli* K12 defective in initiation of DNA replication at high temperature. Mol. Gen. Genet. **110**:299-306.
21. Noack, D., and S. Klaus. 1972. Inactivation kinetics of lambda phage repressors in a mutant of *E. coli* temperature sensitive in DNA replication. Mol. Gen. Genet. **115**:216-224.
22. Sugino, Y., A. Sorai, T. Kogoma, Y. Hirota, and R. Okazaki. 1966. Inhibition of DNA synthesis and induction of Lambda phage in temperature-sensitive mutants of *Escherichia coli*. Jap. J. Genet. **41**:247-249.
23. Wechsler, J. A., and J. D. Gross. 1971. *Escherichia coli* mutants temperature-sensitive for DNA synthesis. Mol. Gen. Genet. **113**:273-284.
24. Wechsler, J. A., V. Nüsslein, B. Otto, A. Klein, F. Bonhoeffer, R. Herrmann, L. Gloger, and H. Schaller. 1973. Isolation and characterization of thermosensitive *Escherichia coli* mutants defective in deoxyribonucleic acid replication. J. Bacteriol. **113**:1381-1388.
25. Worcel, A. 1970. Induction of chromosome re-initiations in a thermosensitive DNA mutant of *Escherichia coli*. J. Mol. Biol. **52**:371-386.
26. Yoshikawa, H., and M. Haas. 1968. On the regulation of the initiation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **33**:843-855.
27. Young, E. T., and R. L. Sinsheimer. 1967. Vegetative bacteriophage λ DNA. I. Infectivity in a spheroplast assay. J. Mol. Biol. **30**:147-164.